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Chronic cannabinoid, CP-55,940, administration alters biotransformation in the rat

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Abstract

The objective of this study was to investigate the effects of single and repeated administration of CP-55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol)} on behaviour, energy metabolism and biotransformation. Single intraperitoneal administration to male Sprague-Dawley rats of CP-55,940 (0.4 mg/kg), induced a behavioural response characterized by 'splayed hind limbs', antinociception, hypothermia and a decrease in locomotor activity. Brain and liver mitochondria of the CP-55,940-treated rats exhibited an increase in respiration and no changes in ADP/O and citrate synthase specific activity. Repeated intraperitoneal administration of CP-55,940 (0.4 mg/kg, 11 days) induced behavioural tolerance, disappearance of the increase in the mitochondrial oxygen consumption as well as an increase in the monooxygenase activities and the content of liver microsomal cytochrome P450. Some hepatic metabolizing enzymes of the cytosolic glutathione-centre system were also affected. Previous studies had indicated that the tolerance after chronic administration of CP-55,940 could be due to down-regulation of brain cannabinoid receptors. The present findings demonstrate that the behavioural tolerance occurs together with modified biotransformation activities.

Keywords: Cannabinoid; CP-55,940; Tolerance; Biotransformation; Energetic metabolism

1. Introduction

The relatively recent identification of a cannabinoid binding site in the central nervous system (Devane et al., 1988) has been accomplished by the use of tritiated CP-55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol)} (Johnson and Melvin, 1986), a synthetic bicyclic cannabinoid compound, which has been used to determine pharmacological, anatomical and molecular characteristics and site distribution of the cannabinoid receptors in various species (Howlett et al., 1990; Herkenham et al., 1990). The specific cannabinoid receptors are presumed to mediate most of the effects of cannabinoid exposure on behavioural processes, including reduction in spontaneous activity, catalepsy, antinociception and hypothermia (Martin et al., 1991). Several animal studies, have demonstrated tolerance to these effects (Compton et al., 1990). When tolerance is demonstrated, there is no indication that the cause of tolerance can be explained based on alterations in the brain and periphery pharmacokinetics. Development of tolerance must thus be described as a pharmacodynamic event rather than a consequence of reduced bioavailability of the active compound. Specifically, Oviedo et al. (1993) showed that animals chronically treated with Δ^9-tetrahydrocannabinol and CP-55,940 had homogeneous decreases in binding in all striatal structures examined, due to a lowering of binding capacity (B_{max}); Rodriguez De Fonseca et al. (1994) confirmed that tolerance was accompanied by significant decreases in the density of cannabinoid receptors in the striatum and limbic forebrain.

In the present work, we have examined whether the behavioural tolerance that developed after 11 days of daily intraperitoneal (i.p.) treatment with CP-55,940 through receptor down-regulation as well as a reduction in levels of cannabinoid receptor mRNA (Rubino et al., 1994), was also partially the result of an increase in drug metabolism.

2. Materials and methods

2.1. Cannabinoid treatment

Male Sprague-Dawley rats (Charles River, Italy) weighing 200–220 g were used. The animals were individually

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housed and had free access to standard laboratory rat chow and water until they were killed.

Rats received i.p. CP-55,940 (generous gift from Dr. D. Casilli, Pfizer Italiana, Rome, Italy) (0.4 mg/kg in 20% ethanol in distilled water) or vehicle. Two different kinds of treatments were used. For the acute treatment, the animals received a single i.p. injection of CP-55,940 or vehicle and were submitted to a behavioural session. For the chronic treatment, a different group of animals received a daily i.p. injection of CP-55,940 (0.4 mg/kg) or vehicle for 11 days. One group of rats always received only distilled water. The animals were submitted to a behavioural testing session on the 1st, 5th and 10th days of treatment. Body weight, fluid and food intake were measured daily.

2.2. Behavioural testing

All the measures of spontaneous locomotor activity and antinociception were obtained in the same animal by measuring, 30 min after the injection, locomotor activity for 5 min and antinociception every 30 min for 4 h. Analysis of locomotor activity was performed by a computer-controlled monitoring system according to the procedure previously described by Rubino et al. (1994) and quantified in terms of the overall distance travelled (cm). Antinociception was assessed using the tail-flick test (D'Amour and Smith, 1941) and was quantified in terms of area under the antinociceptive curve (AUC) expressed as tail-flick latency (s) for 240 min. On the 1st, 5th and 10th days of treatment the number of treated animals showing 'splayed hind limbs' was noted at 30, 60, 90, 120 and 240 min, and this number was expressed as a percentage of the total number of animals treated. These percentages were compared at 60 min, because at this time the other behavioural effects were at their highest level and the animals were killed at this point.

60 min after the last injection, the rats were decapitated and their brains and livers were quickly removed, weighed and used for subcellular fraction preparation.

2.3. Mitochondria

The liver mitochondria were prepared according to the method previously described by Colleoni et al. (1996), and the brain mitochondria were prepared according to the method of Davis et al. (1974). Mitochondrial respiratory rates were determined polarographically using a Clark-type oxygen electrode (Yellow Springs Instruments) at 25°C for liver and 35°C for brain, the solubility of oxygen being taken to be 442 ng atoms/ml at 25°C and 424 ng atoms/ml at 35°C when the medium was air-equilibrated at 760 torr. The reactions were started by adding the rat liver or brain mitochondria (1–3 mg of protein) to the reaction chamber (state 4) followed 2 min later by ADP (560 nmol) in order to produce state 3 (Colleoni et al., 1996). The respiratory rates for states 3 and 4 (expressed as oxygen ng atoms/min/mg mitochondrial protein) and the ADP/O ratios were calculated as described by Colleoni et al. (1996). The respiratory medium was that described by Colleoni et al. (1996) for liver and that of Davis et al. (1974) for brain: for liver, 3 ml of 75 mM KCl, 50 mM Tris HCl (pH 7.4), 12.5 mM K_2HPO_4, 5 mM $MgCl_2$, 1 mM EDTA sodium, 10 mM respiratory substrates and for brain, 3 ml of 0.3 M mannitol, 0.1 mM EDTA sodium, 10 mM KCl, 10 mM Tris HCl (pH 7.4), 1.5 mM K_2HPO_4 (pH 7.4), 0.1% bovine serum albumin, 13 mM glutamate and 6.5 mM succinate as respiratory substrates.

2.4. Liver microsomes and cytosol

The liver microsomes were prepared according to Mitoma's procedure (Mitoma et al., 1956), modified by Uemura et al. (1977). The liver cytosolic fraction was the supernatant after the first centrifugation at $105\,000 \times g$ for 60 min at 4°C. The microsomal pellet and the cytosolic fraction served as the source of microsomal and cytosolic enzymes.

2.5. Enzyme assays

Citrate synthase specific activity of the brain and liver mitochondria (about 26 μg/ml of proteins) was assayed spectrophotometrically according to the procedure described by Colleoni et al. (1996).

Liver microsomal content of cytochromes P450 and b_5 was measured according to Omura and Sato (1964) and Garfinkel (1958), respectively. NADPH cytochrome P450 reductase specific activity was measured by its NADPH cytochrome c reductase activity, according to Omura and Takesue (1970). The following mixed-function oxidase P450-linked activities of the liver microsomes were determined by referenced methods: benzo(a)pyrene hydroxylase (Nebert and Gelboin, 1968) and 7-ethoxycoumarin *O*-deethylase (Greenlee and Poland, 1978). The content of liver cytosolic reduced and oxidized glutathione was measured fluorimetrically according to Hissin and Hilf (1976). Liver cytosolic glutathione *S*-transferase specific activity was measured according to the spectrophotometric procedure described by Santagostino et al. (1989). Liver cytosolic glutathione peroxidase specific activity was measured spectrophotometrically according to Wendel (1981), using both cumene hydroperoxide and H_2O_2 in order to differentiate the glutathione peroxidase Se-dependent activity (H_2O_2) from the non-Se-dependent activity.

2.6. Protein determination

Mitochondrial, microsomal and cytosolic protein concentration was measured by the method of Lowry et al. (1951) with bovine serum albumin as standard.

2.7. Materials and drugs

All chemicals were purchased through normal commercial sources and were of the highest purity available. The cannabinoid, CP-55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol)}, was kindly supplied by Dr. D. Casilli, Pfizer Italiana (Rome, Italy) and was dissolved in 20% ethanol as vehicle. Drug and vehicle were administered i.p. at a volume of 5 ml/kg body weight.

2.8. Statistical analysis

The results are expressed as mean ± S.E.M. Unless otherwise stated, $n = 13$ for the vehicle- and CP-55,940-once treated and $n = 10$ for the vehicle- and CP-55,940-11-days-treated rats. For the incidence of 'splayed hind limbs', statistical analysis was done with Fischer's exact probability test, for the 2×2 contingency table (Zar, 1974). For the mitochondrial results, comparisons between acute/chronic treatment and the respective vehicle were done using Student's *t*-test for paired observations (Zar, 1974). In most cases, mean values were compared using the analysis of variance (ANOVA) followed by Tukey's test (Zar, 1974). A $P \leq 0.05$ was considered statistically significant.

3. Results

The distilled water-treated rats did not differ from the vehicle-treated (20% ethanol in distilled water) rats regarding either behavioural observation or biotransformation parameters.

3.1. Behavioural testing

A single injection of CP-55,940 (0.4 mg/kg i.p.) in rats reduced spontaneous locomotor activity by about 93% (Fig. 1, middle panel). The effect on locomotion disappeared after 11 daily doses of CP-55,940, indicating the development of tolerance to the motor disturbance induced by the cannabinoid. Another significant behavioural effect was observed at this CP-55,940 dose: strong antinociception starting after 15 min and lasting about 4 h.

Tolerance also developed to the antinociceptive effect, antinociception being reduced by about 58% after 10 days of CP-55,940 treatment (Fig. 1, left panel). The area under the antinociceptive curve (AUC) measuring the latency (s) to a tail-flick for 240 min (Fig. 1, left panel), was 840 ± 89 for control rats compared with 3558 ± 440 for CP-55,940-once-treated rats. On the 5th day of CP-55,940 treatment, the AUC of treated rats was 2510 ± 451, and was always significantly different from that of vehicle-treated rats (731 ± 45). The antinociceptive effect disappeared after 10 daily doses of CP-55,940: the AUC of thus treated rats was 1490 ± 96, a result significantly different from the AUC for rats receiving a single dose of CP-55,940 but not from that for vehicle-treated rats (679 ± 28). When placed in the open field, the rats treated once with cannabinoid exhibited a characteristic behaviour, known as 'splayed hind limbs' with an incidence of 60, 80, 100 and 80% after 30, 60, 90 and 120 min from treatment, respectively; after 240 min only 20% was the incidence observed (data not shown). On the 5th day of cannabinoid treatment, 60 min after the injection, only 40% of the treated rats showed 'splayed hind limbs', not more significantly different from vehicle-treated rats (Fig. 1, right panel). On the 10th day of treatment, this behaviour disappeared completely. The value for vehicle-treated rats reported in Fig. 1 was the mean of the 1st, 5th and 10th days of treatment because these values were not significantly different (ANOVA).

The repeated administration of CP-55,940 also induced a decrease (32%) in body weight gain because of decreased food consumption (data not shown); on the 11th day of treatment, the body weight increase of the vehicle-treated rats was 84 ± 6 g and that of the CP-55,940-treated

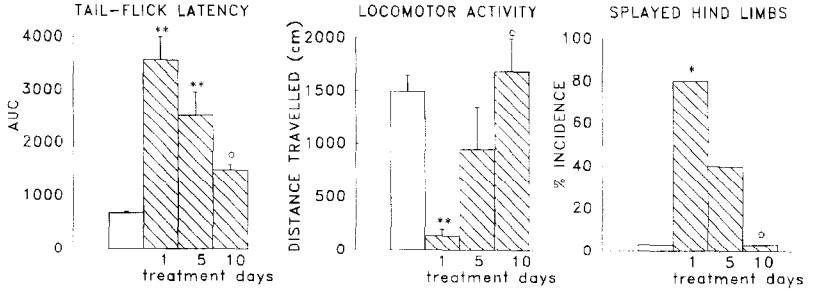

Fig. 1. Effects of prolonged (11 days) administration of CP-55,940 (0.4 mg/kg i.p.) (hatched bars) on behavioural testing. Tail-flick latency (left panel) is expressed as area under the antinociceptive curve for 240 min (AUC) and spontaneous locomotor activity (middle panel) as total distance travelled for 5 min after 30 min. Bar heights represent the mean ± S.E.M. for 4–5 rats. Statistical significance of differences was obtained by ANOVA followed by Tukey's test (** $P \leq 0.01$ as compared with vehicle, ° $P \leq 0.01$ as compared with the 1st day of treatment). Percentage of incidence of the splayed hind limbs (right panel) was evaluated after 60 min. Bar heights represent the percentage incidence for 5 rats (* $P \leq 0.02$ as compared with vehicle; ° $P \leq 0.02$ as compared with the 1st day of treatment according to Fischer's exact probability test for the 2×2 contingency table). The vehicle value (open bars) is the mean of the 1st, 5th and 10th days of treatment because these values were not significantly different.

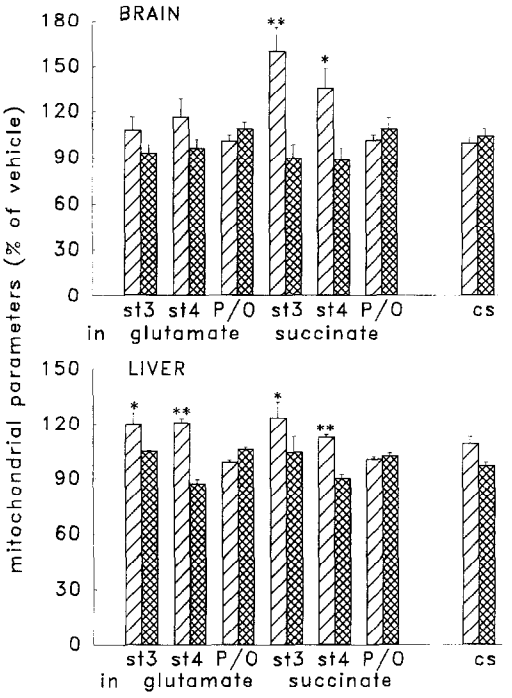

Fig. 2. Effects of CP-55,940 acute (hatched bars) and 11-day (diagonal crosshatch bars) i.p. treatment on rat brain (upper panel) and liver (lower panel) mitochondria. Brain and liver mitochondria were prepared and assayed for respiratory rate in the presence of substrate alone (st4) (glutamate or succinate) as well as for ADP-stimulated (st3) respiratory rate, for the ADP/O ratio (P/O) and for the specific activity of citrate synthase (cs). Bar heights represent the mean ± S.E.M. of the percentages of the values for vehicle mitochondrial parameters, on the 1st and 11th days of treatment. Statistical analysis was performed on mean ± S.E.M. of the mitochondrial parameters from 4–5 rats with Student's *t*-test for paired observations (* $P \leq 0.05$; ** $P \leq 0.01$ as compared with respective vehicle).

rats was 57 ± 4 g; food consumption during 11 days of treatment was 295 ± 17 g for the vehicle-treated rats and 236 ± 7 g for the CP-55,940-treated rats.

3.2. Effects on liver and brain mitochondria

The effects of the prolonged CP-55,940 treatment on the brain (Fig. 2, upper panel) and liver (Fig. 2, lower panel) mitochondrial respiratory rate in the states 3 and 4, on the ADP/O ratio in the presence of two oxidative substrates (glutamate, succinate) and on citrate synthase specific activity are shown in Fig. 2. After a single CP-55,940 treatment the liver oxygen uptake both in states 3 and 4 was significantly increased (approximately 20%); the brain mitochondrial states 3 and 4 were significantly increased only with succinate as oxidative substrate (approximately 60% for state 3, approximately 35% for state 4). The increase in oxygen consumption was not accompanied by either an uncoupling effect (the ADP/O ratio of CP-55,940-once treated rats had the same value as that for the rats treated once with vehicle) or by a decrease in specific activity of citrate synthase, the best marker enzyme for mitochondrial integrity and, more specifically, for the matrix fraction. Our preliminary data showed that CP-55,940, added in vitro at a micromolar concentration (1 μM), elicited a striking increase (more than 100%) in rat brain mitochondrial oxygen consumption during state 3 and 4 respiration with both glutamate and succinate (data not shown). Repeated i.p. administration of CP-55,940 eliminated the increase in brain and liver mitochondria oxygen consumption (Fig. 2, upper and lower panel).

3.3. Effects on liver cytosolic and microsomal enzymes

The liver microsomal contents of cytochromes P450 and b_5 and the mixed-function oxidase P450-linked activities were unaffected (Fig. 3) by a single i.p. administration of CP-55,940. Repeated administration induced a clear increase in both the cytochrome P450 content (34%) and the oxidative enzymatic system: 50% for NADPH cy-

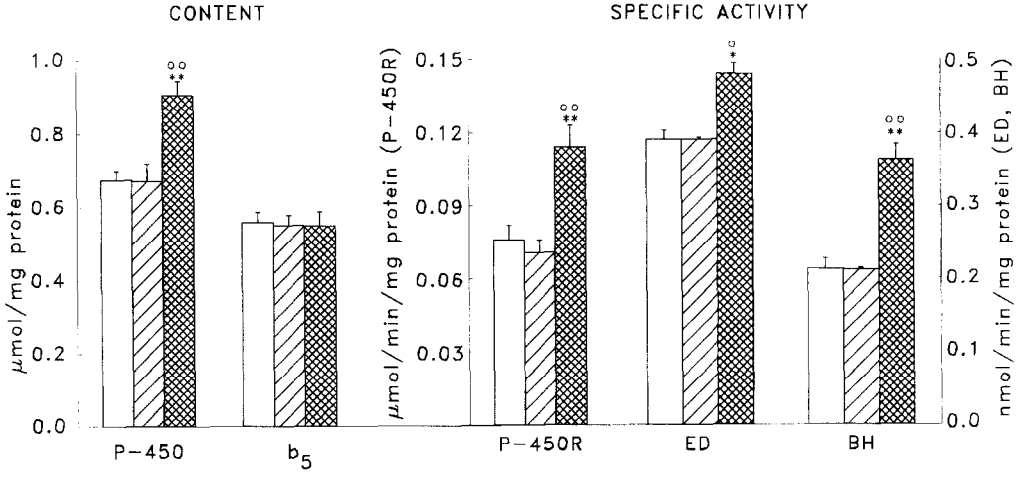

Fig. 3. Effects of CP-55,940 acute (hatched bars) and 11-day (diagonal crosshatch bars) i.p. treatment on rat liver microsomal mixed-function oxidase activity. Hepatic microsomes were prepared and assayed for cytochrome P-450 (P-450) and cytochrome b_5 (b_5) contents and for NADPH cytochrome P-450 reductase (P-450R), benzo(a)pyrene hydroxylase (BH) and etoxycoumarin deethylase (ED) specific activities. Bar heights represent the mean ± S.E.M. of the data from 10–13 rats. The vehicle value (open bars) is the mean of the 1st and 11th days of treatment because these values were not statistically different. Statistical analysis was obtained by ANOVA followed by Tukey's test (* $P \leq 0.05$; ** $P \leq 0.01$ as compared with vehicle; $^{\circ}$ $P \leq 0.05$; $^{\circ\circ}$ $P \leq 0.01$ as compared with the 1st day of treatment).

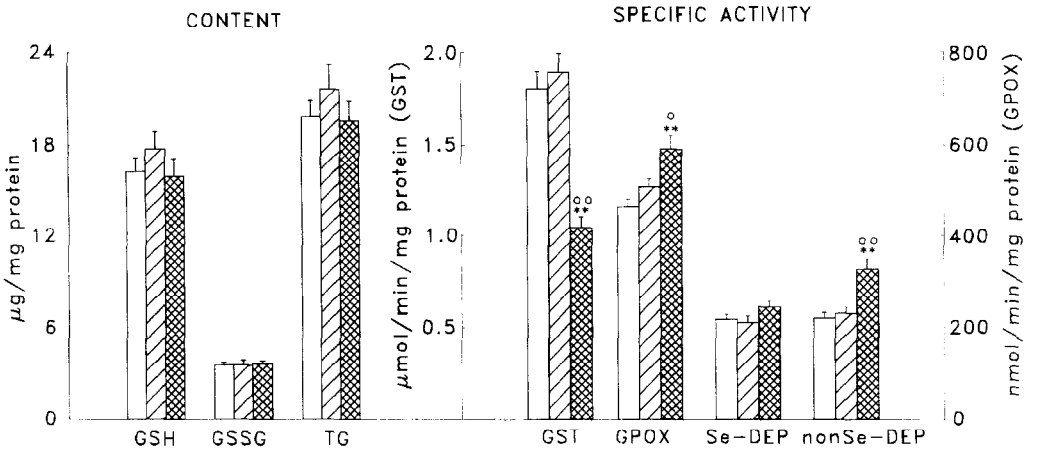

Fig. 4. Effects of CP-55,940 acute (hatched bars) and 11-day (diagonal crosshatch bars) i.p. treatment on liver cytosolic glutathione centre system. Hepatic cytosol was prepared and assayed for the content of total (TG), reduced (GSH) and oxidized (GSSG) glutathione and glutathione *S*-transferase (GST) and peroxidase (GPOX), Se-dependent glutathione peroxidase (Se-DEP) and non-Se-dependent glutathione peroxidase (nonSe-DEP), specific activities. Bar heights represent the mean ± S.E.M. of the experimental data from 10–13 rats. The vehicle value (open bars) is the mean for the 1st and 11th days of treatment because these values were not statistically different. Statistical analysis was done with the ANOVA followed by Tukey's test (* $P \leq 0.05$; ** $P \leq 0.01$ as compared with vehicle; ° $P \leq 0.05$; °° $P \leq 0.01$ as compared with the 1st day of treatment).

tochrome P450 reductase, 23% for 7-ethoxycoumarin *O*-deethylase and 70% for benzo(a)pyrene hyroxylase specific enzymatic activities. Other parameters of liver biotransformation were modified after the prolonged treatment with CP-55,940 (Fig. 4): glutathione *S*-transferase specific activity was significantly decreased (42%), whereas glutathione peroxidase cytosolic specific activity was significantly increased (28%) because of the increase of the non-Se-dependent glutathione peroxidase (48%). The content of total, reduced and oxidized glutathione and the ratio of the reduced to oxidized glutathione content were not modified.

4. Discussion

A number of experimental findings suggest that the behavioural tolerance shown after prolonged i.p. treatment with the cannabinoid, CP-55,940, appears together with modified biotransformation activities. The findings of the present study can be summarized as follows:

(1) The synthetic bicyclic cannabinoid, CP-55,940, is capable of producing pharmacological responses with a profile similar to that seen for Δ^9-tetrahydrocannabinol and other psychoactive natural and synthetic cannabinoids (Pertwee, 1993). At the same dose (0.4 mg/kg i.p.) it produced antinociception and a characteristic posture of the hind legs known as 'splayed hind limbs', as well as depressed spontaneous activity (90%) and a lowering of body temperature by approximately 1°C (data not shown). However, it is well known that CP-55,940 (i.v.) in mice, a species more sensitive to the effects of cannabinoids than is the rat, is more effective to produce antinociception and reduce motor activity than to produce hypothermia (three and nine times respectively, Martin et al., 1991).

(2) Development of tolerance to the behavioural and physiological effects (such as motor effects, hypothermia, hypotension, immunosuppression, weight loss and alterations in schedule-controlled behaviour) has been shown to occur on repeated administration of Δ^9-tetrahydrocannabinol, and other psychoactive cannabinoids (Pertwee, 1991) and recently after chronic exposure to anandamides, the recently discovered endogenous cannabinoid receptor ligands in brain (Fride, 1995). In our study, daily repeated administration of the same CP-55,940 dose also led to progressive behavioural tolerance, the rate depending on the effect considered: only on the 10th day of treatment was tail-flick latency of vehicle- and CP-55,940-treated rats the same. However motor effects and 'splayed hind limbs' disappeared earlier (the 5th day). Tolerance seems to develop more readily to some effects than to others, according to the previous reports by Pertwee (1991). There is little evidence that chronic administration alters cannabinoid disposition or metabolism in the brain (Magour et al., 1977) and periphery, suggesting that tolerance is pharmacodynamic rather than a consequence of reduced bioavailability of active cannabinoids (Siemens and Doyle, 1979; Dewey et al., 1973; Karler et al., 1982). Even though the vehicle-treated rats received 20% ethanol, no alteration in the microsomal drug metabolism was shown. However it is possible that there is a contribution of ethanol to the tolerance induced by CP-55,940 and cross tolerance has indeed been demonstrated between ethanol and Δ^9-tetrahydrocannabinol (Sprague and Craigmill, 1976; Siemens and Doyle, 1979). It seems likely that the cannabinoid receptor may also play a part in the production of cannabinoid tolerance as well as in the mediation of many acute effects of psychotropic cannabinoids. Results of various studies have suggested that chronic administration of Δ^9-tetrahydrocannabinol and CP-55,940 produces behavioural tolerance which has a receptor-mediated basis. The concomitant reduction of cannabinoid receptors available for

binding may be responsible for the behavioural tolerance (Oviedo et al., 1993; Rodriguez De Fonseca et al., 1994; Rubino et al., 1994).

(3) Our study showed that the development of behavioural tolerance to CP-55,940 appears together with modified biotransformation activities; the increase in mitochondrial oxygen consumption induced by a single cannabinoid i.p. administration, disappeared after 11 days injection and the content of liver cytochrome P450 and the linked-cytochrome P450 enzymatic activities were increased. Various and conflicting results have long been reported about the effects of single and repeated Δ^9-tetrahydrocannabinol administration on mitochondrial processes. Nazar et al. (1974) showed that, after acute administration, Δ^9-tetrahydrocannabinol depressed respiration in mouse brain homogenates but after repeated injections was significantly less effective in this regard, indicating acquisition of tolerance to Δ^9-tetrahydrocannabinol. Sprague et al. (1972) demonstrated that chronic i.v. administration of Δ^9-tetrahydrocannabinol increased the respiration of monkey tissue slices from various brain regions. Δ^9-tetrahydrocannabinol in vitro has been reported to uncouple state 4 respiration, to decrease respiratory control and the ADP/O ratio and to release enzymes from the rat liver mitochondria structure. Mahoney and Harris (1972), Lemberger (1972), and Bino et al. (1972) have shown that Δ^9-tetrahydrocannabinol damages the mitochondrial membrane and alters the ATPase activity and oxygen consumption of rat liver mitochondria; Banerji et al. (1985) have shown that Δ^9-tetrahydrocannabinol produces a characteristic stimulation of mitochondria swelling in rat hypothalamus, heart and liver under both in vitro and in vivo conditions. Δ^9-tetrahydrocannabinol and other cannabinoids, including CP-55,940, because of their high lipophilicity (Poddar and Ghosh, 1976) would have strong effects upon membrane-dependent processes: the hydrophobic portion of the cannabinoid molecules would be expected to interact with hydrophobic regions in the membrane and perhaps disturb the functioning of the mitochondrial membrane system (Banerji et al., 1985). The result of the disappearance of the extra stimulation of oxygen consumption induced by a single cannabinoid injection is not per se a sign of metabolic tolerance because CP-55,940 was not measured in tissues. Only if a lower level of CP-55,940 occurred in the brain and liver of animals repeatedly treated with CP-55,940, would the basis of the mechanism of the tolerance to CP-55,940 become apparent. McMillan et al. (1973) failed to find a change in levels of Δ^9-tetrahydrocannabinol and its metabolites in blood and brain tissues (Dewey et al., 1973) of tolerant and non-tolerant pigeons receiving [^{3}H]-Δ^9-tetrahydrocannabinol. Our results regarding the development of tolerance to the respiratory effect after repeated exposure to CP-55,940 were accompanied by an increase in content and enzymatic activity of the liver microsomal oxidative cytochrome P450 system. Other authors (Fernandes et al., 1973; Cohen et al., 1971; Bornheim and Correia, 1989a) have shown that Δ^9-tetrahydrocannabinol and other purified extracts from cannabis induced a substrate-specific inhibition of hepatic microsomal mixed-function oxidases and a decrease in cytochrome P450 content, whereas in our present study, a single CP-55,940 dose had no effect on these microsomal systems. In contrast, enzyme activity is selectively enhanced when CP-55,940 is administered in repeated doses to rats with a concomitant increase in total hepatic cytochrome P450 levels according to Leighty (1979), Mitra et al. (1976) and Bornheim and Correia (1989a). Bornheim and Correia (1989b) have purified and characterized cytochrome P450 isozyme from hepatic microsomes of mice pretreated with cannabidiol; the isozyme exhibited properties characteristic of the major hepatic cytochrome P450 previously purified from phenobarbital-pretreated animals. Khanna et al. (1991) have shown that cannabis is a selective inducer of B-esterases as well as cytochrome P450-dependent oxidative enzymes, such as aromatic hydrocarbon hydroxylase metabolizing benzo(a)pyrene (approximately 3-fold induction). Its interference with metabolism might influence the pharmacological and toxicological manifestations of drugs and other xenobiotics in individuals habituated to the use of cannabis. In spite of the acquired tolerance, CP-55,940 chronic treatment induces some toxicity markers. We have shown a decrease in body weight gain, partially due to a decreased food consumption consistent with that reported by Compton et al. (1990), but different from that reported by other authors who have shown the development of tolerance after repeated Δ^9-tetrahydrocannabinol administration also involving the reduction of body weight (Dewey, 1986). The decrease in liver cytosolic glutathione *S*-transferase specific activity indicates a decreased ability to detoxify the glutathione-conjugates probably formed after CP-55,940 chronic treatment. The increase in non-Se-dependent peroxidase specific activity was a further marker of a toxic situation induced by high concentrations of free radicals.

In conclusion, our findings favour the hypothesis that the mechanism by which CP-55,940, and presumably other cannabinoids, induce behavioural tolerance after prolonged treatment also involves the hepatic drug-metabolizing enzyme system. This conclusion is particularly significant in view of the recent difficulties of Westlake et al. (1991) and Fan et al. (1996) with explaining cannabinoid-induced tolerance only by alterations of brain cannabinoid receptors. We have found direct evidence of an increase in the microsomal cytochrome P450 oxidative system and indirect evidence of the disappearance of the physiological effect on liver and brain mitochondrial respiration.

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